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Adoptive Therapy with T Cells/NK Cells

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ABSTRACT

Graft-versus-tumor effect is well recognized in allogeneic transplantation, but it appears to be disease specific and relapse remains a significant problem. Furthermore, immune reconstitution after hematopoietic cell transplantation is often delayed and incomplete. It is becoming increasingly clear that the immune system is complex and that cooperation between innate and adaptive immunity is required to induce a productive immune response. Progress in clinically applicable cell separation techniques and knowledge of the signals required for effective immune activation have made adoptive therapy with T cells and NK cells a viable treatment option. However, clinical efficacy with either cell type depends on in vivo expansion of the infused product, which is facilitated through mechanisms that are active after lymphodeletion. Although successes have been seen with several approaches, further study of immune biology, lymphocyte cooperation and the role of regulatory T cells will lead to better strategies to exploit adoptive transfer of lymphocytes for therapeutic benefit.

KEY WORDS

Immunotherapy • NK cells • T cells • Vaccines

INTRODUCTION

Immunotherapeutic approaches, including adoptive cellular therapy and vaccines, are playing an increasingly important role in the treatment of malignancies. The principles of adoptive immunotherapy established in animal models have formed the basis for the testing of therapeutic strategies for human tumors. Translating these approaches to the clinic has been difficult due to issues of patient heterogeneity, cost, availability of clinically approved reagents, and the requirement for Good Manufacturing Practice (GMP) facilities. The central premise supporting the use of adoptively transferred lymphocytes, including natural killer (NK) cells and T cells, is that they can exhibit more antitumor activity compared with endogenous lymphocytes. For NK cells, alloreactivity may be increased by donor selection (eg, killer immunoglobulin-like receptor [KIR] ligand–mismatched NK cell donors). For T cells, the ability to apply ex vivo stimuli can expand specific antitumor cells and also may serve to overcome the negative influence of tumors encountered in vivo (eg, T cell anergy). Although it is not the focus of this manuscript, the role of regulatory T cells should be acknowledged, especially given the recent work showing that interleukin (IL)-2 administration may expand regulatory T cells in vivo, which can lead to inhibition of NK cell and T cell function.

The first part of this article, written by Drs. Cooley and Miller, describes the use of NK cell–based immunotherapy. NK cells do not require presensitization for antitumor activity, and allogeneic NK cells do not induce graft-versus-host (GVH) responses, features that make NK cell–based therapies attractive. Current strategies use in vivo expansion of allogeneic NK cells to treat both hematologic malignancies and solid tumors and also to optimize hematopoietic cell transplantation (HCT) protocols by incorporating NK cells. The primary advantage of using adoptively transferred T cells is these cells' ability to specifically target tumor cells that express small peptides even if the intact target protein itself is not expressed on the cell surface. A second advantage is their potentially long clonal lifespan. In

the second part of this article, Dr. June explains the principles of adoptive transfer of T cells and strategies to combine vaccination with adoptive T cell transfer to improve tumor specificity and killing. The success of immunotherapy depends on the generation of adequate numbers of activated effector cells that persist in vivo long enough to exhibit a measurable antitumor response. In the third part of the article, Dr. Schoenberger discusses recent discoveries on the role of $CD4^+$ helper T cells in influencing the development of functional $CD8^+$ cytotoxic T lymphocytes (CTLs). It is becoming increasingly clear that methods of immune activation that orchestrate multiple effectors to work in concert will produce the most effective antitumor treatments. Adoptive transfer of lymphocytes will likely play a significant role in these emerging strategies.

ADOPTIVE TRANSFER AND IN VIVO EXPANSION OF HUMAN ALLOGENEIC NK CELLS TO TREAT CANCER (S. COOLEY AND J. S. MILLER)

Biology of NK Cells

NK cells are effectors of the innate immune system characterized by the expression of CD56 and nonexpression of CD3. They were initially recognized for their ability to lyse virally infected cells and tumors without requiring previous sensitization. They mediate immediate, nonspecific killing of targets in a major histocompatibility complex (MHC)-independent fashion, a property that makes them an attractive cell population to exploit for antitumor immunotherapy. NK cell cytotoxity is regulated by the net balance of activating and inhibitory signals transferred through several classes of receptors, including KIRs [1]. Several of the inhibitory KIRs recognize class I HLA ligands (C1, C2, and Bw4), which protect against NK cell autoreactivity. The absence of class I HLA expression by a target leaves it unable to inhibit the corresponding KIR, rendering it susceptible to lysis by that NK cell.

Early Autologous NK Cell Adoptive Transfer

During the 1980s, several clinical trials investigated adoptive immunotherapy with lymphocytes to treat various malignancies. These early trials were developed to generate autologous lymphokine-activated killer (LAK) cells by ex vivo expansion of peripheral blood mononuclear cells (PBMCs) with IL-2 stimulation for 5 days. Patients were given autologous LAK with high-dose IL-2, and modest success was seen in lymphoma, melanoma, and renal cell cancer, where it was shown that the cytotoxic activity was mediated mainly by NK cells [2]. To decrease complications of vascular leak consistently seen with high-dose IL-2 therapy, several centers tested outpatient low-dose IL-2 administration (± ex vivo IL-2–activated cells) and found that it safely increased the number of activated NK cells circulating in vivo. This approach but did not result in clinical benefit, however. For example, a phase II trial conducted at the University of Minnesota found no efficacy in patients with chronic myelogenous leukemia, lymphoma, or breast cancer [3].

We now understand that the failure of autologous LAK and NK cell therapies likely has been due in part to the down-regulation of NK cell killing by inhibitory KIR recognition of "self" class I MHC on tumor cells. In addition, successful expansion of adoptively transferred lymphocytes depends on adequate lymphodepletion to "clear space" for the infused lymphocytes to compete for growth factors and cytokines. Murine studies have demonstrated effective antitumor immunity after preparative regimens that induced sufficient lymphopenia to allow homoeostatic T cell expansion in vivo [4]. This principle was first confirmed in humans by Rosenberg's group at the National Institutes of Health [5]. T cell lymphopenia was induced by cyclophosphamide (60 mg/kg/day × 2 days) followed by fludarabine (25 mg/m^2/day × 5 days). This Hi-Cy/Flu therapy allowed in vivo expansion of adoptively transferred cytotoxic T-lymphocytes with specificity for melanoma cells, resulting in subsequent clinical efficacy.

Adoptive Transfer and In Vivo Expansion of Allogeneic NK Cells

In attempts to bypass the limitations of autologous NK therapies, we and others have turned to adoptive transfer of allogeneic cells. Alloreactivity may be further increased by selecting donors based on KIR ligand mismatch status with the patient. This strategy predicts that the selection of a donor having a KIR ligand lacking in the patient will correspond to a higher frequency of donor antihost reactive cells. This approach is supported by the work of Ruggeri et al. [6], who showed that in the setting of haploidentical HCT, stratifying patients by their KIR ligand match or mismatch status selected for patients who engrafted with alloreactive NK cells and resulted in improved outcomes for patients with myeloid leukemia. To determine the safety and in vivo expansion of haploidentical related-donor NK cell infusions, we first tested them in a nontransplantation setting, which is safer because permanent engraftment is not expected. Furthermore, because there is less concern for graft-versus-host disease (GVHD), this setting is more amenable to combination therapy with IL-2, which is important for inducing NK cell activation and expansion. We treated 43 patients with either metastatic melanoma (n = 10), metastatic renal cell carcinoma (n = 13), refractory Hodgkin's disease

(n = 1), or refractory acute myelogenous leukemia (AML) (n = 19) [7]. The first 17 patients were treated with a low-intensity outpatient chemotherapy regimen consisting of intravenous cyclophosphamide (750 mg/m^2) and methylprednisolone (1000 mg/m^2) on day −2, followed by NK cell infusions (in escalating doses) on day 0 and IL-2 (1.75×10^6 IU/m^2) daily for 14 days. NK cell expansion was determined using a polymerase chain reaction–based chimerism assay and was considered successful if donor NK cells were measurable by the end of IL-2 therapy. This preparative chemotherapy regimen did not induce lymphopenia on the day of infusion, and, not surprisingly, none of these patients achieved in vivo donor NK cell expansion.

Two other preparative regimens aimed at avoiding NK cell rejection were tested. Seven non-AML patients received intravenous fludarabine (25 mg/m^2) daily for 5 days (days −6 to −2), and 19 AML patients received 1 or 2 doses of intravenous cyclophosphamide (60 mg/kg) and intravenous fludarabine (25 mg/m^2) (Hi-Cy/Flu) daily for 5 days. Although both regimens induced absolute lymphopenia at day 0, the non-AML patients were not neutropenic, and no in vivo NK cell expansion was seen with the low-intensity fludarabine regimen. In contrast, the more intense Hi-Cy/Flu regimen induced pancytopenia, and in vivo expansion of NK cells was seen in 8 of the 15 evaluable patients. A representative donor–recipient pair is shown in Figure 1A, which shows in vivo expansion by the increased band density beyond day 2. The donor NK cells are easily identified as CD56$^+$/CD3$^-$ NK cells in blood (Figure 1B) and are functional in cytotoxicity assays.

In contrast to fludarabine alone, the Hi-Cy/Flu preparative regimen induced a surge of endogenous IL-15 (Figure 1C). An inverse correlation between the absolute lymphocyte count and the IL-15 concentration ($r = -.62$; $P < .0001$) was seen postchemotherapy. The increased IL-15 level after Hi-Cy/Flu therapy may result from gut release due to toxicity induced by cyclophosphamide. Alternatively, the decreasing numbers of mature lymphocytes may lead to decreased utilization of IL-15 and a corresponding increase in plasma IL-15 concentrations. The importance of IL-15 in NK cell homeostasis [8,9] is supported by the correlation between high levels and successful NK expansion.

Clinical Efficacy Correlates with In Vivo NK Expansion and KIR Ligand Mismatch

Five of 19 patients with poor-prognosis AML achieved complete remissions after the Hi-Cy/Flu regimen and NK cell infusion. Compared with patients who failed to clear leukemia, the patients in remission had significantly higher proportions of circulating NK cells (35% ± 8% vs 1.5% ± 0.4%; $P = .001$) and cytotoxicity against K562 targets (50% ± 10% vs 10% ± 5% at E:T 20:1; $P = .01$). These findings support the conclusion that allogeneic donor NK cells expand in vivo and contribute to efficacy. Although it was not a donor selection criterion, we evaluated the KIR mismatch status in the AML cohort. Four of 19 patients exhibited alloreactivity based on KIR ligand mismatch in the GVH direction. Interestingly, 3 of 4 (75%) patients with KIR ligand mismatch, but only 2 of 15 (13%) patients with KIR ligand match, achieved complete remission. Although our study sample is small, these data support a role for KIR ligand mismatching or other strategies in promoting alloreactivity to treat AML.

The NK products in this initial trial were prepared from single lymphapheresis collections using a GMP immunomagnetic CD3 depletion. The final products, after overnight activation in IL-2, contained a median NK cell dose of 9.4×10^6 cells/kg (range, 4-13 $\times 10^6$ cells/kg) (n = 36). We thought that contaminating T cells might compete for NK cell expansion, and that B cells might lead to Epstein-Barr virus (EBV)-driven lymphoproliferation; therefore, we modified the process to include a CD56 selection step, resulting in increased NK cell purity (90% ± 7% CD56$^+$/CD3$^-$ NK cells compared with 38% ± 13% with CD3 depletion alone), and minimal contamination with CD19$^+$ B cells or CD14$^+$ monocytes. However, no NK expansion was seen in the first 10 patients treated with the purified NK cell products. This failure to expand may be due to NK cell loss with more extensive processing, where approximately 1/3 the NK cell recovery was seen compared with CD3 depletion, resulting in an NK cell dose of 3.4×10^6 cells/kg (range, 0.15-6.6 $\times 10^6$ cells/kg). Alternatively, the contaminating B cells and monocytes removed in the 2-step depletion strategy may serve critical roles in NK cell activation or expansion. For example, we have shown that contact with autologous monocytes is important for in vitro NK cell expansion [10]. We have returned to CD3 depletion alone for the NK products in the ongoing trial in AML.

We are currently studying adoptively transferred allogeneic NK cells in several other settings. We are using the Hi-Cy/Flu preparative chemotherapy to investigate NK cell expansion and antitumor activity in patients with metastatic breast cancer, a disease known to be responsive to NK cell killing in vitro. In addition, we are testing 2 strategies for incorporating allogeneic NK cell therapy with HCT. The presence of alloreactive NK cells during the early recovery phase of a traditional transplantation protocol may contribute to more potent antileukemic therapy, may increase the tolerability of the preparative regimen by inducing more rapid neutrophil recovery, and may provide prolonged immune therapy. Besides decreasing relapse

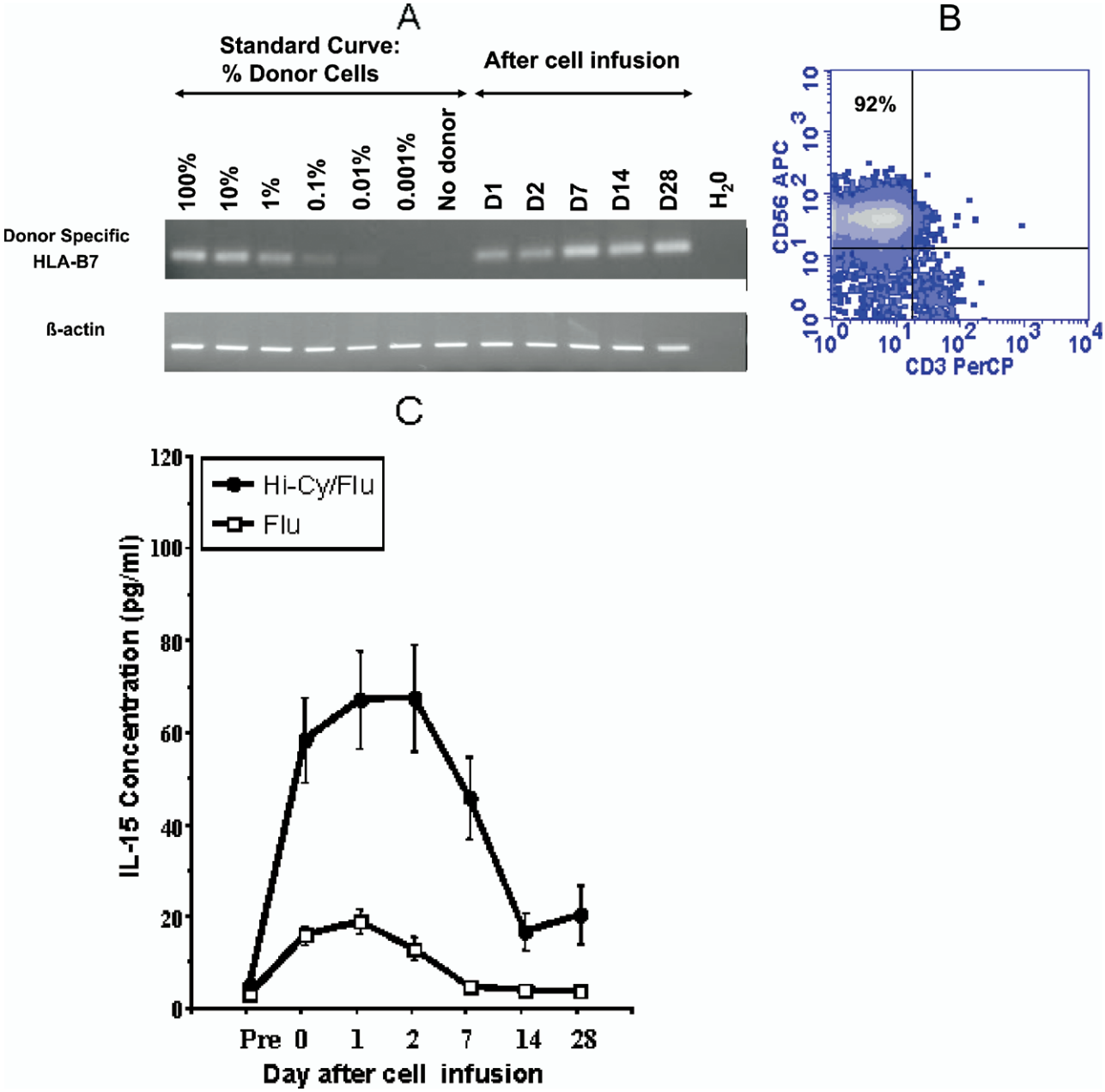

Figure 1. In vivo expansion of haploidentical NK cells correlates with high endogenous IL-15 levels induced by Hi-Cy/Flu. (A) A standard curve was established between donor and recipient cells by polymerase chain reaction amplification of an HLA allele unique to the donor (percentage of donor cells shown above). Postinfusion recipient samples were tested for the presence of the unique donor HLA allele. In this example, donor NK cells were detectable at day 2 and had expanded by day 7, as evidenced by the increased band density. The β-actin control demonstrates equal sample loading. (B) PBMCs were collected from patients at day 14 after haploidentical NK cell infusion and lymphocyte subsets were measured by flow cytometry. For the patient shown here, 92% of all circulating cells were $CD56^{+}/CD3^{-}$ NK cells with a female karyotype in this male patient. (C) Plasma was collected from patients before and after the indicated preparative regimens. The day 0 sample was collected before the NK cell infusion. The plotted lines represent the mean (± the standard error under the mean) of samples from each cohort. IL-15 concentrations, measured by enzyme-linked immunosorbent assay, were markedly higher in patients who received Hi-Cy/Flu compared with those who received Flu alone.

rates by mediating direct antileukemia killing, NK cells can improve outcomes after HCT by decreasing the incidence of GVHD and increasing engraftment [6]. We are testing these hypotheses in patients with poor-prognosis AML by infusing allogeneic NK cell products derived from haploidentical related donors or from umbilical cord blood (UCB) before standard haploidentical or UCB HCT after nonmyeloablative versus fully ablative preparative regimens, respectively.

Therapeutic Limitations of NK Cells

The therapeutic potential for adoptive transfer of allogeneic NK cells has several limitations. Lymphapheresis collections can produce only limited numbers of NK cells. Although we have shown that in vivo NK cell expansion is possible, its success remains unpredictable, and the durability of the expanded cell population is limited. In vivo expanded NK cell populations are heterogeneous, expressing variable KIR repertoires that produce variable alloreactivity. Along with KIR ligand mismatch status, the antitumor efficacy of NK cell therapy depends on the expression of the appropriate activating ligands on the tumor cells. For example, the lack of efficacy of KIR-mismatched transplants in lymphoid leukemias may be due to their low expression of LFA-1 or NKG2D ligands. Future strategies to enhance activating ligand expression on leukemia cells is needed to make them more susceptible to NK cell–mediated lysis.

Future Directions

Numerous strategies to address these limitations are under active investigation. Irradiated cell lines such as NK92 and KHYG-1 may provide an inexhaustible supply of highly cytotoxic NK cells, but they are IL-2–dependent and have very short in vivo survival. Techniques for ex vivo expansion of adult NK cells are currently being refined by several groups. Alternatively, large numbers of NK cells may be derived from UCB sources [11]. UCB-derived NK cells also may exhibit superior in vivo expansion. Ex vivo expanded cells from any source can be genetically modified to express tumor-specific receptors. For example, the NK92 cell line has been transfected with a chimeric antigen receptor for HER2/neu, which conferred superior cytotoxicity against HER2/neu-positive targets [12]. Refinements in the administration of lymphodepleting chemotherapy may optimize the cytokine milieu for NK cell expansion. Furthermore, selective depletion of regulatory T cells, which can suppress NK cell proliferation and killing, also may improve the immune effector functions of the expanding NK cells [13]. NK cells may be made more alloreactive by using antibodies to block inhibitory KIR [14] or more tumor reactive by targeting NK cell antibody-dependent cell-mediated cytotoxicity with tumor-specific monoclonal antibodies, such as rituximab or trastuzumab. Many investigators have demonstrated the role of allogeneic NK cells in the treatment of various malignancies, either through adoptive transfer or as part of HCT. Ultimately, improvements in techniques to generate adequate numbers of alloreactive NK cells and to make tumor cells more susceptible to their effects will increase the clinical efficacy of NK cell–based immunotherapy.

COMBINING VACCINES WITH ADOPTIVE IMMUNOTHERAPY AND PERIPHERAL BLOOD STEM CELLS (C. H. JUNE)

Principles of T Cell Adoptive Transfer

Studies conducted over the past several decades have uncovered a number of principles of adoptive immunotherapy using T cells, including to (1) avoid the induction of immunogenicity of the infused cells, (2) prevent or delay cellular immunosenescence, (3) maximize $CD4^+$ T cell help, and (4) induce host lymphopenia with conditioning chemotherapy. Recent studies show that the in vivo persistence and engraftment of tumor-infiltrating lymphocytes correlates with telomere length and tumor regression in patients with melanoma [15]. Many studies show that the generation and/or maintenance of $CD8^+$ memory requires $CD4^+$ cell help, [16] and clinical adoptive transfer studies have shown that the persistence of cytotoxic $CD8^+$ effector T cells (CTLs) is enhanced with the concomitant administration of IL-2 or $CD4^+$ cells. Finally, host lymphodepletion may enhance the effectiveness of adoptively transferred T cells [4]. Lymphodepletion eliminates regulatory T cells and other competing elements of the immune system that act as "cytokine sinks," enhancing the availability of cytokines such as IL-7 and IL-15 [17]. This hypothesis has been tested clinically in patients with metastatic melanoma and myeloma, where initial results suggest that the efficacy of adoptive transfer is improved in patients rendered immunodeficient by cytotoxic chemotherapy [5,18].

Approaches for Adoptive T Cell Therapy

Two basic approaches to T cell adoptive immunotherapy are currently being tested. The first approach involves isolating and activating antigen-specific T cells from peripheral blood or tumor specimens and then clonally expand those antigen-specific cells in vitro by various approaches [19]. In the second approach, the patient is primed with a vaccine before lymphocyte harvest, followed by polyclonal ex vivo activation of the input T cells, based on the assumptions that antigen-specific T cells are present and that they have been primed in vivo (Figure 2). The first approach will guarantee antigen specificity but is costly and labor-intensive. The second approach is technically more facile. Both approaches have been promoted by the realization that homeostatic expansion of transferred lymphocytes can improve engraftment and effector functions in vivo [4].

Strategies to Combine Therapeutic Vaccination and Adoptive T Cell Therapy

Therapeutic vaccination of patients is likely to succeed mainly in the setting of minimal residual disease and probably will require priming and booster

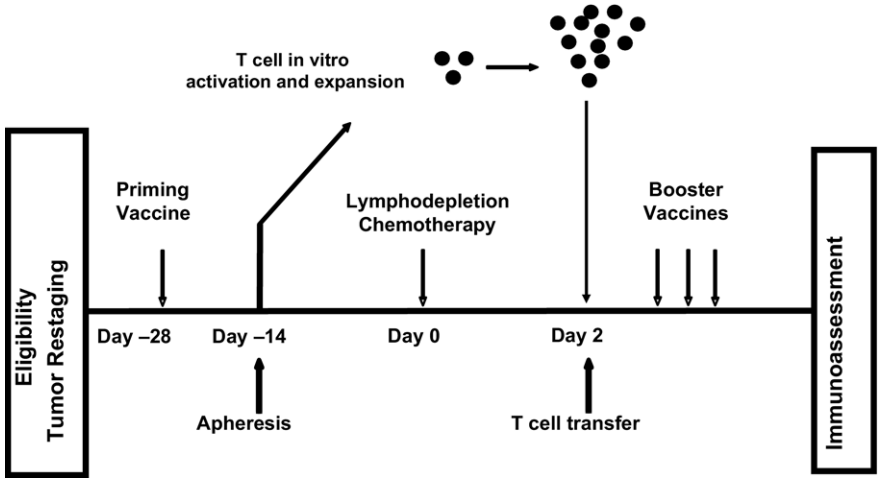

Figure 2. Schema for adoptive transfer of autologous, vaccine-primed, in vitro–expanded T cells. As shown here, eligible patients are primed with vaccine on day −28, followed by lymphocyte harvest on day −14. The autologous T cells undergo polyclonal in vitro activation and expansion and are reinfused on day 2 after lymphodepleting chemotherapy. Antigen-specific immune function is measured after the administration of booster vaccines.

vaccinations to improve or maintain immunity. A corollary to this assumption is that therapies combining vaccination with other biologic agents with distinct but complementary mechanisms of action are required to achieve maximum benefit. In mice, vaccines and adoptive transfer of cells in the setting of lymphopenia results in enhanced tumor immunity [4,20,21]. In humans with myeloma, idiotype vaccination of sibling donors followed by adoptive transfer in the form of allogeneic stem cell transplantation can result in the induction of potent tumor immunity [22]. Although this approach is theoretically attractive, at present there are only limited data in humans demonstrating the efficacy of a combined vaccine and adoptive T cell transfer approach in the autologous setting.

Two phase I trials using autologous activated or "costimulated" T cells in patients undergoing transplantation for hematologic malignancies have been reported. In the first trial, patients with relapsed or chemotherapy refractory non-Hodgkin's lymphoma were treated with $CD34^+$-selected HCT followed by infusion of autologous T cells stimulated with anti-CD3 and anti-CD28 [23]. Infusion of autologous costimulated T cells resulted in a rapid reconstitution of lymphocyte counts. Importantly, the expanded cells were functionally superior to those obtained directly from the patients, as determined by interferon-gamma induction. Complete or partial responses were observed in 8 of the 17 patients receiving infusion. Building on the previous trial, the authors next examined the role of pretransplantation immunization and T cell add-back in autologous transplantation for multiple myeloma [18]. All patients received 2 doses of Prevnar, the 7-valent pneumococcal conjugate vaccine (PCV), beginning 1 month after transplantation. Half of the patients received an additional dose of Prevnar 2 weeks before cells were harvested for the transplant. The harvested T cells were expanded in vitro. Patients received a standard, non–lymphocyte-depleted, autologous transplant after melphalan conditioning and then received costimulated expanded autologous T cells either 14 days or 100 days posttransplantation. Prompt T cell recovery was observed in both patient groups that received T cell add-back on day 14, whereas the day-100 add-back groups remained significantly lymphopenic. Only those individuals who received PCV-primed T cells early after transplantation developed and maintained protective levels of antipneumococcal antibodies, as well as PCV-specific CD4 responses. These data demonstrate that combination vaccine and adoptive immunotherapy comprising a single pretransplantation vaccine and an early posttransplantation infusion of antigen-primed, ex vivo costimulated autologous T cells followed by posttransplantation booster immunizations decreased the severe immunodeficiency associated with high-dose chemotherapy and led to clinically relevant immunity in adults within a month after transplantation. Although larger trials are needed, this pilot study provides a useful foundation for future strategies using combinations of tumor vaccines and costimulated T cells in lymphodepleted patients with lymphoma or other hematologic malignancies.

Shu and Chang have developed an alternative approach using tumor-infiltrating lymphocytes that should circumvent many of the limitations posed by adoptive transfer. They and others have shown in mice that tumor–draining lymph nodes harbor T cells that are not capable of mediating tumor rejection in adoptive transfer experiments. In contrast, if the draining lymph node cells are activated in vitro, then the cells gain the capacity to mediate tumor rejection

after adoptive transfer. In further studies, T cells were isolated from vaccine-primed lymph nodes obtained from patients with melanoma, renal cell carcinoma, and head and neck cancer. In the absence of antigen-presenting cells, activation with anti-CD3 and anti-CD28 greatly enhanced subsequent T cell expansion in IL-2 (> 100-fold) compared with anti-CD3 alone [24]. Based on these and other preclinical studies, Chang and coworkers carried out a phase I trial in patients with late-stage melanoma and renal cell carcinoma [25]. Patients were given intradermal vaccination with irradiated autologous tumor cells. The draining lymph nodes were harvested 7-10 days later, at which point the vaccine-primed T cells were cultured and infused. Among the 11 patients with melanoma, 1 had a partial tumor response; the 12 patients with renal cell carcinoma included 2 complete and 2 partial responses. Thus there may be some clinical activity with this approach, and it could be adapted for hematologic malignancies, particularly for lymphoma therapy.

With the exception of idiotype and EBV-induced lymphoproliferative disease, an obstacle to combined vaccine and adoptive T cell therapy of hematologic malignancies is that the optimal tumor antigens remain undefined. A unique and truly antigen-specific target is the idiotype antigen receptor present on many lymphomas and myelomas. Idiotype has proven to be a robust target for vaccine strategies for patients with follicular lymphomas [26], and in preclinical models, T cells have been shown to target idiotype [27]. However, clinical attempts to target the idiotype with adoptively transferred T cells have not yet been successful. A more widely applicable form of cellular therapy could be developed with the identification of universal tumor antigens that are (1) presented by most MHC types, (2) expressed and presented in most tumors, (3) expressed in limited amounts in normal tissues, and (4) directly involved in the malignant phenotype of the tumor. Recent studies have identified several candidates for universal tumor antigens, including the catalytic subunit of telomerase (hTERT), cytochrome P450 isoform 1B1, survivin, proteinase 3, WT-1, and MDM2.

Adoptive T Cell Therapy: Dose and Scheduling Issues

Information on the dose and schedule dependence of adoptively transferred T cells is widely scattered in the literature, and no standardized dose system has been described. Schedule-dependent efficacy and adverse effects from adoptively transferred cells have been reported. There is evidence in nonlymphopenic hosts suggesting that fractionated doses of adoptively transferred T cells are superior to a single infusion of T cells [28]. The ideal dose of transferred cells is related to the tumor burden and to the homing and persistence (memory) characteristics of the infused cells. Doses of adoptively transferred cells are usually reported as the total number of viable cells administered or as the total number of viable cells per kg body weight or per square meter body surface area. However, total lymphocyte numbers do not correlate well with body surface area and in fact display a stronger inverse correlation with age. Other variables add to the complexity, particularly when using T cells or other adoptively transferred cells with high replicative potential. The infused dose may not relate well to the steady-state number of cells. Therefore, dosage considerations are more complex than in other areas of transfusion medicine where, for example, the maximal level of transfused red cells or platelets occurs immediately after infusion. Recent studies of adoptively transferred autologous $CD4^+$ T cells found that the highest number of cells in the host peaked 2 weeks after cell infusion [29]. This is because the engraftment potential and the replicative potential of the infused cells depends on complex host variables, such as the number of niches available in the host for engraftment and the antigenic stimulus for clonal expansion or deletion [17]. In most rodent tumor models, T cell proliferation in the host after transfer is obligatory for therapeutic efficacy [30].

Many studies in rodent tumor models have shown that the coadministration of cytotoxic therapy can enhance the effects of adoptively transferred cells. Currently, cyclophosphamide and fludarabine are the preferred agents. The mechanism is likely due not to cytoreduction, but rather to multiple other effects, including (1) the killing of host regulatory lymphocytes that suppress antitumor immune responses, (2) the creation of "space" in the host so that the adoptively transferred cells can engraft, and perhaps (3) enhanced cross-priming of tumor antigens. Curti et al. [31] examined a related issue concerning the optimal time for harvesting autologous T cells in relation to the timing of cyclophosphamide administration in patients with lymphoma and other advanced cancers. T cells were harvested at steady state, when declining, or when recovering after cyclophosphamide-induced leukopenia. These authors concluded that the best time to harvest autologous T cells was not at steady state, but rather just before the leukopenic nadir after cyclophosphamide administration. The optimum in vivo expansion of the infused $CD4^+$ T cells occurred when the cells had been harvested as patients entered the cyclophosphamide-induced nadir. Most of the clinical antitumor responses also occurred in patients treated on this schedule. These results are generally consistent with animal models that predicted a need to ablate immunosuppressive lymphocytes for efficient engraftment and subsequent in vivo expansion of adoptively transferred $CD4^+$ T cells.

In patients with early-stage cancers who have not yet received cytotoxic chemotherapy, it is probably

best to harvest autologous T cells before initiation of chemotherapy. This recommendation is based on the limited regeneratative capacity of T cells from the adult thymus. After chemotherapy, the repertoire remains contracted for long periods, and in many cases it never recovers. Naive T cells are most sensitive to the effects of cytotoxic chemotherapy, and their numbers are severely depleted in heavily pretreated patients. It is not yet known whether the antitumor specific T cells are derived from primed or naive T cells in the host; this likely varies depending on the intrinsic immunogenicity of the tumor.

Summary

It is likely that adoptive immunotherapy will not be used alone, but rather will be combined with vaccines, chemotherapy, and other forms of immunotherapy. Based on the pioneering work of Kwak and Levy [32], idiotype-specific vaccines for patients with follicular lymphoma are likely to be approved soon, providing an important opportunity to combine therapeutic vaccines with adoptive T cell transfer therapy.

THE TRAIL FROM T HELPER TO CD8$^+$ T CELL MEMORY (S. SCHOENBERGER)

The successful clinical application of adoptive immunotherapy will require a clearer understanding of the cellular and molecular pathways governing the response of key effector populations. Recent studies have provided new insight into the role of CD4$^+$ helper T cells in influencing the signals that control the functional development and survival of MHC class I–restricted CD8$^+$ CTLs [16]. The focus of much of this work has been on determining how the signals transmitted antigen-presenting cells after activation by CD4$^+$ helper T cells are integrated into the CTL response. These signals induce CTLs to (1) undergo primary expansion, (2) acquire effector function, and (3) give rise to a subset that become bona fide memory cells that are endowed with 2 additional functional capacities: enhanced survival and secondary expansion on reencounter with antigen. Several lines of evidence indicate that each of these fates is directed largely by an instructional "program" that is set into motion by inductive signals received during priming of clonal precursors, and is then carried out by their postexpansion progeny [33]. Identifying the essential elements of this program, along with the signals and molecular pathways through which it is encoded and executed, will provide the information needed to generate and maintain clinically beneficial CTL responses in adoptive therapy. A necessary first step is to define the parameters of CTL function influenced by the presence or absence of CD4$^+$ T cell help.

Help for Secondary Expansion

The effectiveness of a given population of antigen-specific CTLs depends on the capacity of selected clones to expand their numbers through division. Although the primary burst may increase the initial frequency of a given clone by 100- to 1000-fold or more, these cells will undergo a contraction phase during which 90%–95% of their numbers are lost [34]. The capacity of these primary CTLs to undergo a second round of division should be considered a crucial aspect of functional immune memory, because it allows the primary clonal progeny to reach the higher frequencies needed to deal with a substantial target cell population, such as might exist within a tumor or virally infected organ. It was noted by 4 groups, all working with distinct antigenic systems, that a central function of CD4$^+$ T cell help is to endow CTLs with the capacity for secondary expansion after restimulation in vitro and in vivo [16]. Studies using physiological numbers of precursors have demonstrated that the help appears to confer the functional capacity for secondary expansion during the first 1-2 days of priming. This finding is consistent with a role for CD4$^+$ T cells in modifying the instructional program of CTL development to include secondary expansion [35]. In contrast, adoptive-transfer models studying high numbers of antigen-specific transgenic CTLs after an acute infection have shown that CD4$^+$ T cells appear to exert their influence over a longer period. Curiously, this "maintenance" capacity does not appear to require antigen specificity [36].

Help for Survival?

The observation that CTLs acquire the capacity for secondary expansion through an early modification of their developmental program that occurs during priming in the presence of help was the basis for the hypothesis that the "helped" cells would follow a specific pattern of gene expression distinct from their "helpless" counterparts. To document this, we followed the fate of helpless CTLs after restimulation. In contrast to the helped cells that undergo secondary expansion, we found that the helpless CTLs undergo activation-induced cell death (AICD). The apoptosis is mediated by the expression of TNF-related apoptosis inducing ligand (TRAIL)/apo2L, a member of the TNF superfamily of death receptor/ligand pairs [37-39]. TRAIL expression was found to be regulated by selective transcription in the helpless cells, although antigenic stimulation induced expression of the TRAIL receptor (TRAIL-R2/DR5) in both helped and helpless CTLs. This raises the possibility that memory CTLs can be made to undergo TRAIL-R-mediated AICD on restimulation [37]. Notably, the TRAIL produced by

the restimulated helpless cells is in a soluble form, suggesting that it may be able to exert an immunoregulatory effect on neighboring T cells or even on antigen-presenting cells [40].

Future Perspectives

This information regarding $CD4^+$ T cell help and the role of TRAIL in the regulation of CTL responses sheds new light on questions critical to the field of adoptive immunotherapy and suggests future avenues of investigation. The identification of the signals that direct the differentiation of CTLs toward the helped pathway during primary activation remains an important problem. Understanding the mechanism through which these inductive stimuli are "hard-wired" into CTLs, preserved throughout clonal expansion, and integrated into the response of the CTL progeny will offer a novel perspective on the molecular regulation of differentiation. Finally, the idea that helpless CTLs produce a soluble form of TRAIL on antigenic stimulation raises the possibility that such cells can be enumerated through an enzyme-linked immunospot assay, which would provide a direct, useful phenotypic measurement of T cells in identifying populations for use in adoptive immunotherapy.

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